Immune Mechanisms of Gram-Negative Infections

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Life-threatening and fatal infections with gram-negative bacteria have become increasingly frequent during recent years in spite of an overall reduction in mortality from other bacterial infections.[5, 35, 42] This increase has resulted from a combination of factors including a rise in the number of geriatric patients who frequently have chronic and debilitating diseases, more complex and lengthier operations, a greater use of antibacterial agents effective against gram-positive organisms but relatively ineffective against gram-negative organisms, and an increased utilization of therapeutic agents that suppress host resistance. Since alterations of the host resistance so obviously contribute to the development of gram-negative infections, it is timely to review the immune mechanisms that normally afford protection against infection by these bacteria.

The clinical course of gram-negative infections will not be discussed here since this has been reviewed recently,[5, 26, 27] and many clinical aspects of gram-negative infections are considered elsewhere in this symposium. Only those facets of inflammatory processes and immune mechanisms which seem to be particularly pertinent to an understanding of the dynamics of gram-negative infections in humans will be reviewed. While a number of references are cited, it would be virtually impossible to list all of those relevant to any of the subjects under discussion. For the interested reader, three books may be of particular value.[47, 53, 54]

GENERAL DESCRIPTION OF THE IMMUNE RESPONSE

Once bacteria gain entrance into the extracellular tissues of the human host, they provide a series of stimuli for the production of an

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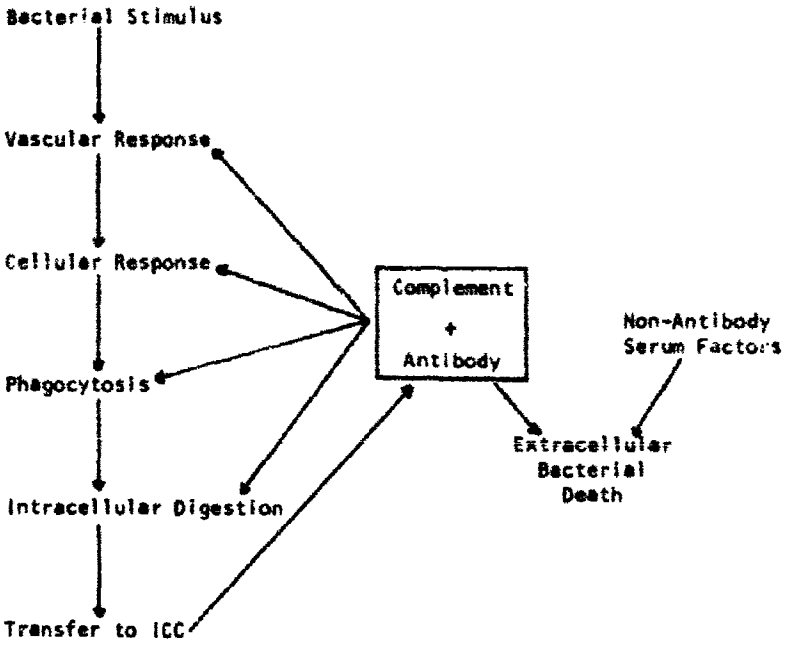

Figure 1. The host defense mechanisms against gram-negative bacteria.

inflammatory focus which are somewhat dependent upon the characteristics of the invading organism. The interactions between the bacteria and the host defense mechanisms determine the presence or absence of overt clinical infection as well as the severity of any infection which may ensue. While these interactions may vary due to differences in bacterial species, the defense mechanisms against gram-negative bacteria are generally similar and will be discussed together (Fig. 1).

Both intracellular and extracellular bacterial products, including endotoxin, may initiate a vascular response by causing direct injury of the capillary endothelium or by producing constriction of the venular sphincters at the junction of the collecting venules and small veins which in turn results in increased venular and capillary pressures. A change occurs in the endothelium that causes adherence of leukocytes which then emigrate through the intercellular spaces into the extravascular tissues. Once outside the capillary lumen, phagocytes may attack the bacterial cells by ingestion and subsequent intracellular digestion. Certain phagocytes have the ability to transfer antigenic materials to immune competent cells, primarily lymphocytes, so that specific antibody against the invading organism may be made. Specific antibody and complement are important components of the host defense mechanism against bacterial infection and may by themselves produce bacterial death without the aid of phagocytosis, particularly with certain species of gram-negative organisms. Serum factors not involving antibody or complement are relatively unimportant. Each of these phases of the immune mechanism will be discussed separately, and it will be shown that a block may occur at any of these separate phases which could serve to aid bacterial invasion.

BACTERIAL CHARACTERISTICS

Escherichia coli is the most common organism recovered from patients with clinical infections due to a gram-negative bacillus, but infections with Proteus, Pseudomonas, and Aerobacter occur commonly.[5, 28] Less frequent are infections with Bacteroides, Paracolobactrum, Achromobacter, and Alcaligenes. Bacteroides is often present in mixed infections, but it is not often recovered by routine culture since strict anaerobic techniques are not utilized in most clinical laboratories.

These organisms often differ considerably in their bacteriologic characteristics, but they share many common features. The typical syndrome recognized as gram-negative septicemia may be produced by each. Furthermore, each is primarily of endogenous origin; each usually is felt to be of low or moderate virulence; and infections with these organisms occur primarily in patients with impairment of their basic defense mechanisms, such as those being treated for cancer with chemotherapeutic drugs, following radiation therapy, in the newborn infant, in patients treated with steroids, and in a variety of other conditions occurring either as a result of natural disease or as an adverse effect of therapy.

Most gram-negative organisms produce an endotoxin-like material which is highly toxic, and other bacterial products have been isolated from individual bacteria such as hemolysins, lecithins, and proteases which produce an adverse effect upon the host. The strain difference in virulence between smooth and rough forms of the same organism has long been recognized, but only recently has it been shown that the surface characteristics of the smooth strains have an anticomplement activity which contributes significantly to its virulence.[53]

THE VASCULAR RESPONSE

The vascular response is an integral part of the host defense mechanism. It is brought about by a variety of mediators, both of endogenous and exogenous origin, and the primary beneficial effects result from the delivery of phagocytes and plasma proteins to the site of a bacterial focus. Anything that impedes the normal vascular response will result in a decreased delivery of phagocytes and plasma proteins with resultant promotion of bacterial infection.

The introduction of gram-negative bacteria into normal tissues, as well as many other noxious stimuli, elicits a response characterized by venular constriction, venular and capillary dilatation with increased venular and capillary pressure, extravasation of plasma proteins, alteration of the endothelial membrane with adherence of leukocytes to the endothelium, and emigration of these leukocytes through openings at intercellular junctions.[54] The initial vascular response may be mediated through a variety of chemical stimuli including endotoxin, histamine, serotonin, bradykinin, and other polypeptides that produce an immediate response. Other permeability factors have been described, such as

Hageman factor, kallikrein, permeability-producing globulin, and factors derived from certain tissue extracts that are not dialyzable. These are discussed in detail elsewhere[9, 15, 16, 21, 23, 39, 46] and will not be elaborated upon except to say that any or all may be involved in the production of increased vascular permeability and emigration of leukocytes induced by bacterial contamination. Hurley[23] has emphasized that the permeability and emigration of leukocytes often coexist, or are subsequent events, and scarcely can be separated.

THE CELLULAR RESPONSE

The first investigator to describe the sticking and emigration of white cells was Dutrochet in 1824.[11] Since that time, a large number of investigators have studied the emigration of leukocytes at inflammatory foci, but many facets of this problem remain unsolved.[54] It is not known whether the adherence of leukocytes to endothelium at a site of injury may be attributed primarily to changes in the endothelium or in the leukocyte, but it is probably the former which is altered first. There is a suggestion that the activation of a proteolytic enzyme is an essential step leading to leukocyte emigration as it may be inhibited by sodium salicylate and by soy bean trypsin inhibitor. Once a leukocyte becomes sticky, it retains this property, indicating that the process is not immediately reversible. Phagocytes enter the extravascular tissues by diapedesis at the intercellular junctions between the endothelial cells. Lymphocytes do not exit in this manner, but rather go directly through the endothelial cytoplasm.

Once outside the blood vessel, locomotion of phagocytes[54] occurs in an apparently random method. Under ideal conditions, neutrophils may travel 35 to 40 microns per minute, usually in a zigzag path. The mechanism for locomotion in the phagocyte is poorly understood, although it involves the gel-sol state of the cell membrane. Divalent cations are required for adherence of a neutrophil to a particle although other substances such as plasma proteins or enzymes may play an important role. Phagocytes move about randomly in an inflammatory focus, but once a neutrophil gets within a certain range of an attractive object such as a bacterium, chemotaxis plays an important role, and the cell stops progressing in a random pattern and proceeds in a reasonably straight path toward the attractive particle. This phenomenon is probably related to a chemical concentration gradient and usually is not effective over distances greater than 100 microns. Chemotaxis by antigen antibody complexes requires the action of complement, but other particles apparently do not.

According to Hirsch,[54] 20 to 30 billion neutrophils are circulating in the blood stream of a healthy man at any one time and an equal number of mature cells are felt to be marginated on vessel walls or sequestered in shutdown capillaries. The half-life of neutrophils is less than a day, and once they emigrate into tissues their life span is terminated there. For every circulating neutrophil, 50 to 100 mature cells

are held in the bone marrow reserve. It is estimated that 50 to 100 ml of packed neutrophils are eliminated daily by a normal person, most of them into the intestinal or respiratory tracts.[54]

During the earliest phases of mild inflammation, monocytes can be observed to leave the vasculature by means of the intercellular spaces, similar to neutrophils.[54] Volkman and Gowins[46] have shown in experiments with rats that mononuclear cells present at sites of inflammation are derived primarily from monocytes which have their origin in the bone marrow. Monocytes live considerably longer than neutrophils—a fact which explains their apparent increase in numbers at the site of inflammatory foci as the lesions age. The preponderance of mononuclear phagocytes and/or eosinophils in certain types of early inflammatory reactions has not been adequately explained.[54]

THE HUMORAL RESPONSE

Humoral factors without an accompanying cellular response are relatively unimportant in the defense of a host against infection. By far the most important humoral components are specific antibody and complement. Alone, neither has bactericidal capabilities, but together may kill many gram-negative bacteria *in vitro*.[33, 53] The extracellular antibacterial activity of antibody and complement has not been determined *in vivo*, although it appears that similar events take place. Both antibody and complement are vital to the processes of phagocytosis and intracellular digestion, and these aspects will be discussed later.

Properdin was described by Pillemer et al.[34] in 1954 to be a normally occurring serum substance with antibacterial activity particularly against gram-negative organisms. Later workers, however, have felt that properdin represents small quantities of specific 19S gamma globulin which, acting with complement has a destructive effect for gram-negative bacteria.[20, 38]

Natural antibody, a name given to gamma globulin with a broad spectrum of activity, probably represents a mixture of antibodies made in response to specific antigenic stimuli since its activity may be specifically absorbed.[12] The enteric bacteria are virtually ubiquitous and omnipresent, and it hardly seems surprising that the so-called natural antibody against gram-negative organisms is present in the sera of normal individuals.

Lysozyme is found in high concentrations in sweat, tears, and saliva and in lower concentrations in serum and other body fluids. It acts synergistically with antibody and complement in the lysis of a wide range of gram-negative and a few gram-positive bacteria.[20, 31, 53]

PHAGOCYTOSIS

The process of phagocytosis, like chemotaxis, is incompletely understood,[36] but it obviously involves an alteration of the cell membrane. During phagocytosis, the cytoplasmic membrane flows around

the object until it is finally engulfed by this invagination, analogous to pinocytosis. Neutrophils engulf particles efficiently over a pH range of 6 to 8, and the process is not dependent upon oxygen. Energy is derived from glycolysis, and phagocytosis is an active process which requires divalent ions.[54] Allison et al.[4] found that epsilon aminocaproic acid effectively blocked phagocytosis and that low levels of trypsin activity accentuated phagocytosis. They were able to postulate that enzymatic activity was necessary for the process. Phagocytosis occurs poorly in suspension, and fibular structures such as fibrin markedly enhance the process.[47]

Particle uptake by neutrophils, monocytes, and fixed reticuloendothelial cells is strikingly dependent upon the presence of serum factors, notably antibody and complement.[38, 51] So-called normal or natural opsonins have been found by Vaughn[44] to have specific activity in that they may be specifically absorbed from sera with particles of the same nature as those used for phagocytic testing. Interestingly, he found that the opsonic activity of normal serum for certain nonspecific substances such as polystyrene particles and zymosan granules could be specifically depleted by prior adsorption. It would appear that opsonic activity is dependent upon antibody adsorbed to the surface of particulate substances, and that this antibody may be adsorbed either nonspecifically or as a result of chemical similarities between the adsorbing substance and an antigen to which the antibody was formed. Opsonic activity of normal serum for certain substances, therefore, appears to be a happenstance. Complement will also influence the phagocytosis of inert particles such as starch.[54]

Specific antibody of high titer markedly promotes the phagocytic process by coating bacteria with antibody. If bacteria are in sufficient numbers so that aggregation occurs, phagocytosis is further promoted since particle clearance is related directly to particle size. Opsonic antibodies are related only to somatic antigens and not to flagellar antigens.[20, 41, 53]

Complement has been found to play an integral role in phagocytosis and is essential for full phagocytic activity.[36, 44] Antibodies to complement will block phagocytosis even in the presence of specific immune opsonin, and inactivation of complement in immune sera will markedly decrease the opsonic activity of that sera *in vitro*.[44] Mucin will inhibit phagocytosis, probably because of anticomplement activity of the substances.[25] It is noteworthy that the phagocytic activity of macrophages is much higher than that of neutrophils in the absence of sera in *in vitro* systems.[44] The effect of both specific antibody and complement is to increase the rate of ingestion. Virtually any manipulation which results in reticuloendothelial blockade is associated with an increased susceptibility of the animal to bacterial challenge, at least temporarily.

INTRACELLULAR DIGESTION

Once bacteria have been phagocytized by either granulocytes or macrophages, intracellular destruction takes place in most instances.

Certain types of bacteria such as Brucella, Salmonella, and Mycobacterium often successfully resist intracellular destruction and may persist in the cytoplasm of these phagocytes in a viable state for long periods of time. Specific antibody not only promotes phagocytosis but also enhances intracellular digestion,[38] and complement serves a similar function. This is particularly true of gram-negative bacterial species.

In one rather elegant study by Wilson, Wiley, and Bruno,[52] an electric current was used to disrupt leukocytes after the ingestion of bacteria. It was found that streptococci that resided inside neutrophils for less than five minutes almost always survived and multiplied whereas those organisms held within neutrophils for 15 minutes or longer were killed. Morphologic changes in the gross appearance or staining properties of ingested microbes in neutrophils usually occurred after 30 to 60 minutes, and the process continued to fragmentation and complete digestion over the course of several hours.

Degranulation accompanies phagocytosis[7] and is related to the emptying of the digestive enzymes confined to the granules (the lysosomes) into the sac surrounding the ingested bacterium (the phagosome).[54] At least 12 acid hydrolases are known to exist in lysosomes of various cell types.[10, 45, 50, 54] Bacterial destruction is imparted by a reduction of the pH to as low as four or five and by the introduction of organic acids, lysozyme, phagocytin, leukin, peroxide, etc. from the lysosome into the phagosome. Lysozyme is a substance of low molecular weight which destroys the cell wall and causes lysis of some species of bacteria.[38] Acting with other agents such as antibody and complement, it kills many other microbes as well.[20, 31, 53, 54] It has been found in a concentration of approximately 2 mg. per gram of packed neutrophils.[50] Phagocytin is associated with the globulin fraction of granules of leukocytes and is relatively heat-stable at acid pH.[19] It is not dependent upon divalent ions for its action, and exhibits a bactericidal pH optimum in the acid range. Its effect is mostly against gram-negative organisms, although some activity has been demonstrated against gram-positive bacteria.[20] Spitznagel and Zeya[40] have described a highly bactericidal, basic, arginine-rich protein derived from the cytoplasmic granules of neutrophils which becomes fixed to phagocytized microbial cells and tissues, at which time the microbial cells are killed. They were able to separate this material from lysozyme, ribonuclease, and deoxyribonuclease by electrophoresis.

Antibacterial fractions occur less frequently in macrophage cytoplasm, but bacterial killing does take place although it is at a much slower rate than in neutrophils.[41] Neutrophils are the primary agents of defense in the destruction of bacteria at an inflammatory focus, whereas fixed macrophages are the primary defense in protection against infection by gram-negative organisms introduced into the general circulation.

TRANSFER TO IMMUNE COMPETENT CELLS

The transfer of antigenic information derived from phagocytized bacteria to immune competent cells is relatively poorly understood.

It is felt that ribonucleic acid,[6, 13] with or without antigenic material derived from degradated bacteria, may be transferred directly from the phagocytic to immune competent cells in the regional lymph nodes and elsewhere in the body through cytoplasmic bridges between the phagocytes and the immune competent cells.[47] Since small lymphocytes are found at the sites of acute inflammation, it is reasonable to assume that this transfer of information occurs there as well as in the regional lymph nodes. Once antigenic information has been obtained by the lymphocyte, the antibody forming mechanism is initiated and specific antibodies against the invading organism are produced. Other antigens, degradated by intracellular enzymes, may be released in a soluble form at the time of death and disintegration of neutrophils.[47]

SUPPRESSION OF IMMUNE MECHANISMS AGAINST GRAM-NEGATIVE INFECTION IN SPECIFIC CONDITIONS

Disease entities that are associated with a diminished resistance to infection invariably are associated with a block in one or more phases of the immune mechanism.

Conditions that impair the vascular response and thus the delivery of leukocytes and plasma proteins to an infectious nidus have been found to enhance infection. The occurrence of infection in contaminated wounds containing devitalized tissues or foreign bodies is so frequent that no emphasis need be given except to point out that the reason for the susceptibility to infection is a lack of delivery of phagocytes to the contaminated focus. Miles and Niven[29] have shown that local host defense mechanisms are sharply suppressed by the injection of epinephrine at the site of bacterial contamination in cutaneous lesions. That the induction of shock to the point of impaired cutaneous perfusion has a similar effect has been shown by the same investigators and by a cooperative clinical study on wound infections by five university centers.[30] Thrombosis of small vessels or decreased perfusion, such as occurs in thermal injury, will similarly promote bacterial proliferation. When bacteria are introduced on suture materials which are of a braided rather than a monofilament nature, the occurrence and severity of infection are increased,[3] presumably because phagocytes cannot enter into the interstices of multifilament suture materials whereas bacteria may do so and can proliferate therein. The resistance of granulating wounds to infection by a variety of bacteria also demonstrates the importance of the ability of the host to deliver living phagocytes to areas where bacteria have gained access to the tissues. Healing wounds exhibit a progressively increased resistance to bacterial infection during the first week after surgical injury,[1] and this increase in resistance to infection parallels the increase in vascularity and cellular reaction. The susceptibility of patients with agranulocytosis, nitrogen mustard therapy, irradiation, or other leukopenic conditions in which neutrophilic production or function is depressed, clearly shows that the neutrophil is an integral unit in host resistance. Inhibition of the production of phagocytes by any means will promote the development of infection.

Other clinical conditions occur in which phagocytes may be present in normal numbers but are abnormal in their ability to phagocytize bacteria or to digest bacteria once they have been phagocytized. These conditions include certain malignancies,[37] prematurity,[16] diabetes,[24] and fatal granulomatosis of childhood (Good's disease). The last disease is a hereditary condition, carried as a sex-linked trait, which is characterized by a susceptibility to severe infections. In this condition there is a lysosomal defect, and phagocytes are unable to digest bacteria in a normal fashion, resulting in the occurrence of granulomatous lesions to stimuli by most types of bacteria. Endotoxin preparations by intravenous injection depress the migratory power of phagocytes for 6 to 12 hours, during which time the host is susceptible to infection. Cortisone causes a stabilization of the lysosomal membrane which interferes with the fusion of the lysosomal membrane and the phagosomal membrane surrounding ingested bacteria and hence with the emptying of the lysosomal contents into the phagosome.[54]

Nutritional deficiencies have been implicated occasionally as a cause of an increased susceptibility to infection, but starvation must occur to the point of inanition and vitamin depletion before nonspecific resistance and the capacity to synthesize antibodies are impaired.[53]

It is well recognized that infectious complications occur frequently in persons with congenital hypogammaglobulinemia. The importance of specific gamma globulin as an opsonizing and cytotoxic agent for gram-negative bacteria has been emphasized.

Depletion of serum complement levels may result in an increased susceptibility to infection, particularly of the gram-negative variety. Complement levels in various disease conditions have not been studied adequately, but several conditions have been shown experimentally to deplete complement or have an anticomplement activity. In these conditions, infection is promoted. The administration of large amounts of heparin or mucin locally with bacteria has an anticomplement effect which markedly enhances the development of infection,[25] and hemoglobin may have a similar effect.[37] The systemic administration of an anticomplement substance, sodium polyanethol sulfonate (Liquoid), will result in an enhancement of the development of cutaneous infection.[28] In patients with severe thermal injury, complement has been found to be decreased,[14] and may contribute to the increased susceptibility to infection seen immediately following thermal injury. Blockage of the reticuloendothelial system by inert carbon particles has been found to increase susceptibility to bacterial infection, and probably does so by depletion of complement inasmuch as restoration of complement to normal levels will abolish the blockade.[44]

ENHANCEMENT OF THE IMMUNE RESPONSE AND ITS CLINICAL APPLICATION

The most obvious application of the aforementioned information is to avoid specific conditions which depress the immune response in any of its phases. Adherence to long-established surgical principles in

most instances complies with adherence to recently developed immunologic principles. Hypotension during operation is to be avoided not only because of the deleterious effect upon the neurologic, cardiovascular, and renal systems but also because decreased perfusion of bacterially contaminated areas promotes infection. Similarly, avascular tissues, epinephrine injection, and foreign bodies must not be combined with bacterial contamination if infection is to be avoided. The use of the principle of delayed primary closure in contaminated wounds will prevent the development of infection when properly and judiciously applied.

Passive administration of homologous pooled gamma globulin is beneficial for a variety of conditions, but for the prevention of infections its usefulness is limited primarily to those individuals who are unable to manufacture their own antibody, as premature infants or patients with congenital hypogammaglobulinemia. The administration of specific antibody for the prevention or therapy of certain gram-negative infections is as much as 20,000 times more effective on a weight basis than is the administration of pooled gamma globulin. While production of specific antibody for specific gram-negative infection has not been utilized widely in the past, the production of an effective antigen for protection against infection with Pseudomonas aeruginosa has been accomplished.[2] Success with a program of active or passive immunization against Pseudomonas aeruginosa may act as a prototype for the development of vaccines against other gram-negative infections. Experimental evidence is encouraging, and clinical studies in human patients are now in progress.

Thermolabile complement components have not been administered clinically, but should deserve consideration in specific conditions.

Stimulation of phagocytic activity has been accomplished by a number of mechanisms. Killed bacilli of Mycobacterium tuberculosis, zymosan, and small doses of endotoxin have been shown to increase the phagocytic activity of the reticuloendothelial system and enhance resistance to infection. Many factors may be involved, including multiplication of macrophages, increased activity of individual macrophages, and release of opsonic factors into the circulation. Administration of thyroid hormone increases general metabolism, including the reticuloendothelial system, and also increases protection from infection.[53] Recently, Nicol et al.[32] have demonstrated that certain estrogenic substances are strong reticuloendothelial stimulants, and they postulated that this may be the natural stimulant of body defense. This finding may explain the known greater susceptibility of male individuals to bacterial infections.[49] Studies in which estrogenic substances have been given to patients for the prevention of systemic infection have not been reported. However, recent experience with the birth control pills has shown their administration to be beneficial in the treatment of acne and furunculosis. Leukocyte stimulation occurs as a natural event in many bacterial infections, but the mechanisms are largely unknown, and there has been little endeavor to study mechanisms by which leukocyte production may be increased as a means of treating naturally

occurring bacterial infections. It has been shown, however, that the passive transfer of living leukocytes to individuals with a depressed leukocyte response may be beneficial in the treatment of certain acute bacterial infections.[37]

Vitamin A has been found to produce a relative instability of the lysosomal membrane. A depletion of vitamin A has been found to enhance infection.[43] It is possible that the induction of hypervitaminosis A might be used to enhance the resistance against bacterial infection in patients with an increased adrenocortical function.

SUMMARY AND CONCLUSIONS

A general description of natural defense mechanisms acting against gram-negative infections has been presented with a discussion of the processes by which bacterial infection may be enhanced or suppressed. The importance of the cellular defense mechanism has been emphasized with an attempt to demonstrate the important interrelationships with humoral defense mechanisms. It is believed that an understanding of the basic physiologic processes involved in the normal evolution of the immune response will benefit the surgeon in the rational avoidance and therapy of specific infections.

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